REMARKS

Each rejection raised by the Examiner is addressed separately below. In view of the additional information and arguments discussed below, Applicants respectfully request reconsideration of the merits of this patent application.

IN THE CLAIMS

Claims 60, 61, 63, 64, 66 and 67 are pending in this application. No amendments have been made, and no new matter has been added.

REJECTIONS UNDER 35 U.S.C. § 103

Claims 60, 61, 63, 64, 66 and 67 are rejected under 35 U.S.C. 103(a) as being unpatentable over Roche Molecular Biochemicals Catalog, 1999, pages 50-51 ("Roche"); Sellman et al. Journal of Bacteriology, Vol 174, No. 13, pages 4350-4355 ("Sellman") ; and Lu et al. BioFeedback, Vol 11, No. 4, pages 464-466, 1991 ("Lu").

The Examiner characterizes Roche as teaching that *Carboxydothermus hydrogenoformans* DNA polymerase has magnesium-dependent reverse transcriptase activity, and opines that, since Sellman and Lu teach that Bst DNA polymerase and Bst DNA polymerase have optimal DNA-dependent DNA polymerase activity in the presence of magnesium ions, it would have been obvious to test for the reverse transcriptase activity of Bst DNA polymerase in the presence of magnesium ions in the absence of manganese ions.

The Examiner goes on to acknowledge (second paragraph, page 3 of Examiner's February 27th, 2009 response) that "it is appreciated that the skilled artisan would understand that different enzymes from different sources would have different activities, properties or optimum reaction conditions." However, the Examiner alleges that because Roche teaches that manganese has a negative effect on fidelity of DNA synthesis and Spellman teaches that Bst DNA polymerase requires Mg2+ for optimal activity, one of skill in the art would have been motivated to test whether Bst DNA polymerase has reverse transcriptase activity in the presence of Mg2+ and in the absence of Mn2+. Applicants respectfully disagree with this line of reasoning for two reasons.

First, as further discussed below, it was and still is widely known that virtually all DNA polymerases have DNA-dependent DNA polymerase activity in the presence of Mg2+ and in the absence of Mn2+. However, it was and still is widely known that most DNA polymerases do not have reverse transcriptase activity in the presence of Mg2+ and in the absence of Mn2+. Thus, the fact that Spellman and/or Lu teach that Bst DNA polymerase requires Mg2+ for optimal DNA-dependent DNA polymerase activity is irrelevant to whether or not a particular DNA polymerase has reverse transcriptase activity or to whether or not such reverse transcriptase activity, if it exists at all, requires Mn2+.

Second, as further discussed below, specific and widely known information in the art concerning the reverse transcriptase activity of Bst DNA polymerase taught that Mn2+ ions were required for such reverse transcriptase activity. In contrast, the present claims recite methods for preparing Bst DNA polymerase holoenzyme or a Bst DNA polymerase large fragment for reverse transcription of RNA molecules in the presence of Mg2+ ions and in the absence of Mn2+ ions. Even though Bst DNA polymerase is widely known to one of skill in the art, references in the art at the time of the present invention specifically teach that Bst DNA polymerase had reverse transcriptase activity only in the presence of Mn2+ ions.

For instance, Bst DNA polymerase was purified as early as 1972 (Stenesh, J and Roe, BA, Biochim. Biophys. Acta 272: 156-166, 1972) and both the holoenzyme and large fragments of Bst DNA polymerase have been cloned (e.g., see U.S. Patent No. 5,814,506 to Kong et al.). Over the years, forms of Bst DNA polymerase have found widespread use in various molecular biology applications, such as in DNA sequencing and for different DNA amplification methods wherein its strand displacement DNA polymerase activity was found to be beneficial. However, to the best of Applicants knowledge, in spite of the long period during which one of the forms (i.e., a holoenzyme or a large fragment) of Bst DNA polymerase has been used, the first experimental demonstration that Bst DNA polymerase has reverse transcriptase activity appears to have been in U.S. Patent No. 6,030,814 to Jerome J. Jendrisak ("Jendrisak") which issued on February 29, 2000. (Jerome J. Jendrisak is Vice President of Research and Development at Epicentre Technologies, of Madison, Wisconsin, the assignee of the present application).

Jendrisak clearly teaches that Bst DNA polymerase, like Tth DNA polymerase, is a manganese-dependent reverse transcriptase. For example, in the Abstract Jendrisak states:

> "A method of improving the synthesis of full-length cDNA transcripts by Mn++ -dependent reverse transcriptases, preferably DNA-dependent DNA polymerases, is disclosed." (*emphasis added*).

Jendrisak goes on to show that addition of betaine to the reaction mixture improves the reverse transcriptase activity of Tth DNA polymerase and Bst DNA polymerase when manganese is included in the reaction mixture. For example, in the fifth paragraph of the Detailed Description of the Invention, Jendrisak states:

> "The examples below describe a typical reverse transcription reaction mixture. A preferred reaction mixture includes RNA template molecules, oligonucleotide primers, a mixture of all four dNTPs, and a suitable buffer. The examples below disclose the use of a buffer comprising 0.01 M Tris-HCl, pH 8.3, 0.05 M KCl, 1.5 mM $MgCl_2$ and 0.75 mM $MnCl_2$. U.S. Pat. Nos. 5,322,770; 5,310,652; and 5,407,800 describe Mn-dependent reverse transcription reactions." (*emphasis added*).

Still further, the only independent kit claim of Jendrisak (Claim 7) claims:

"7. A kit for reverse transcriptase reaction comprising a container of betaine-containing solution, an aliquot of manganese and instructions for the reverse transcription process."

Clearly, Jendrisak did not envision that Bst DNA polymerase would function as a reverse transcriptase in the absence of Mn2+ ions, as is taught by the present invention. Thus, one of skill in the art at the time of the invention would have been aware of references in the art such as Jendrisak which only taught that Bst DNA polymerase had Mn++ -dependent reverse transcriptase activity. At the time of the present invention, one of skill in the art would not have

known that that Bst DNA polymerase might have reverse transcriptase activity in the presence of Mg2+ ions and in the absence of Mn2+ ions.

In short, neither Roche, nor Sellman or Lu, either alone or in combination, teach or suggest the subject matter of the pending claims. The pending claims recite the DNA polymerase from *Bacillus stearothermophilus (Bst)* type strain 5 having reverse transcriptase activity in the absence of manganese ions. Nothing in Roche, Sellman or Lu teach or suggest that replacing the enzyme of Roche with the *Bacillus stearothermophilus (Bst)* type strain 5 polymerase of the pending claims, a polymerase known at the time of Applicants invention to have reverse transcriptase activity only in the presence of manganese ions, will be feasible, let alone successful. Accordingly, Applicants respectfully submit that the pending claims are not obvious over Roche in view of Sellman and/or Lu. Withdrawal is requested.

CONCLUSION

The application is believed to be in condition for allowance and allowance of the same is requested. If all the claims are not allowed, Applicant requests a telephone interview with the Examiner and his supervisor. The Commissioner is authorized to charge any fees under 37 CFR § 1.17 that may be due on this application to Deposit Account 17-0055. Applicants have enclosed a Petition for Two Month Extension of Time and a Request for Continued Examination. If further fees are necessary, please charge Deposit Account 17-0055. The Commissioner is also authorized to treat this amendment and any future reply in this matter requiring a petition for an extension of time as incorporating a petition for extension of time for the appropriate length of time as provided by 37 CFR § 136(a)(3).

Respectfully submitted,

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